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# Is Hepatitis G/GB Virus-C Virus Hepatotropic?
## Detection of Hepatitis G/GB Virus-C Viral RNA in Liver and Serum

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The recently identified hepatitis G virus (HGV, also named GB virus-C, GBV-C) appears to have similarities to hepatitis C virus and other flaviviridae. To better understand its clinical significance and hepatotropism, we collected liver tissue and matched serum samples from 56 patients undergoing liver transplantation. HGV/GBV-C RNA was detected by reverse transcription–nested PCR, using primers from the relatively conserved 5′ noncoding region of the genome to detect HGV/GBV-C RNA and the amount was semiquantitatively estimated by serial 10-fold endpoint dilution. The presence and amount of HCV RNA was estimated by the same methodology. Seventeen patients (30%) had HGV/GBV-C RNA detectable either in liver or in serum, including two of three with cryptogenic liver disease. Interestingly, 5 of 17 (29%) patients had HGV/GBV-C RNA in serum but not liver, even with repeated testing of hepatic RNA from different portions of the liver. Furthermore, the titer of HGV/GBV-C RNA was significantly lower in liver than in serum in most samples (mean log titer, 1.33 vs. 2.56, $P < 0.05$). In contrast, all 21 patients with HCV RNA in serum also had the virus detectable in liver. In five patients coinfected with HCV and HGV/GBV-C, the mean titer of HCV RNA in liver was higher than that in serum (log titer, 2.8 vs. 3.0, $P > 0.05$). Thus, our results suggest that HGV/GBV-C is probably not hepatotropic and may replicate predominantly in sites other than the liver. These findings brings into question the role of HGV in causing significant liver disease. ***J. Med. Virol. 58:160–164, 1999.*** 

**KEY WORDS:** cirrhosis; hepatotropism; hepatitis C; hepatitis G

## INTRODUCTION

The hepatitis G virus/GB virus-C (HGV/GBV-C) is a recently discovered viral agent cloned from the serum of individuals with hepatitis [Simons et al., 1995; Linnen et al., 1996]. It has an RNA genome approximately 10,000 base pairs in length, which is similar in overall structure to that of other flaviviridae. In fact, HGV has approximately 20% nucleotide homology with the hepatitis C virus (HCV), the major etiologic agent of bloodborne non-A, non-B hepatitis. Like HCV, HGV/GBV-C appears to result commonly in chronic infection. Since its discovery, the prevalence of HGV/GBV-C in different population groups has been extensively surveyed [Berg et al., 1996; Alter HJ et al., 1997; Alter MJ et al., 1997; Dickson et al., 1997; Dussol et al., 1997; Francesconi et al., 1997; Hwang et al., 1997; Nakao et al., 1997]. The relationship of HGV/GBV-C infection with liver disease and possible interaction between HGV/GBV-C and known hepatitis viruses have also been estimated in many studies [Yoshiba et al., 1995; Berg et al., 1996; Tanaka et al., 1996; Alter HJ et al., 1997; Francesconi et al., 1997; Martinot et al., 1997; Tagger et al., 1997]. However, so far no clear link has been established between HGV/GBV-C infection and chronic liver disease.

Thus, although HGV/GBV-C RNA has been isolated from serum of patients with acute or chronic hepatitis or cirrhosis, it is also found in serum of controls with other forms of liver disease or no liver injury at all. We therefore studied the presence of HGV/GBV-C RNA within liver tissue and compared it quantitatively in both serum and liver in comparison to HCV, a well-known hepatotropic virus, with the aim of determining if HGV/GBV-C was hepatotropic or not.

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## MATERIALS AND METHODS

We studied 56 patients undergoing liver transplantation in Saint Louis University Hospital between 1993 and 1997. Anti-HCV was tested in all patients routinely by second-generation enzyme-linked immunosorbent assay (ELISA) to make a clinical diagnosis of hepatitis C. Liver tissues were obtained at the time of total hepatectomy, immediately snap-frozen in liquid nitrogen and then stored at –70°C until used. Matched serum samples also were collected, centrifuged immediately after clot formation, and stored similarly. This study was approved by the Institutional Review Board of Saint Louis University and patients gave written informed consent to participate.

Total cellular RNA was extracted from liver as described by Chomczynski and Sacchi [1987] with minor modifications. Briefly, the frozen tissue (approximately 60 mg) was homogenized in 500-μl denaturing solution containing 4-M guanidinium thiocyanate, 2-mM sodium citrate (pH 7.0), 5% sarcosyl, and 0.1-M mercaptoethanol. Sequentially, 50 μml of 2-M sodium acetate (pH 4.5), 500-μl water-saturated phenol, and 200-μl chloroform-isoamyl alcohol mixture (49:1) were added and mixed. The suspension was placed on ice for 15 min and then centrifuged at 14,000 g at 4°C for 20 min. The aqueous layer was extracted and precipitated with two volumes of absolute ethanol at –20°C overnight. The RNA pellet was washed twice in 70% ethanol and resuspended in 80-μl DEPC-treated autoclaved water containing RNAsin (200 U/ml $H_2O$) (Promega, Madison, WI). The amount of RNA was determined by spectrophotometry. For serum RNA, 50-μl serum was first precipitated with 200-μl PBS and 250 μl of 16% PEG at 4°C overnight, centrifuged and the pellet dissolved in denaturing solution as described above. HGV/GBV-C RNA and HCV RNA were detected by reverse transcription–polymerase chain reaction (RT-PCR).

To detect HGV RNA, primers designed by EuGene primer design software (version 1.01) from the 5′ UTR region of the HGV genome were used. The sequences of the outer primers were GF1, sense, nucleotides 131–156, 5′-GGTGGGTAGGTCGTAAATCCCGGTCA3′; GR1, antisense, nucleotides 392–373, 5′-TCCTTGTCAACTCGCCGGCT3′. The inner primers were as follows: GF2, sense, nucleotides 160–180, 5′-TGGTAGCCACTATAGGTGGGT3′; GR2, antisense, nucleotides 352–371, 5′-RGCCTATTGGTCAAGAGRGA3′, where R = A + G. The nucleotide number is based on the sequence of GenBank Accession U44402. In order to avoid cross-contamination, a single tube assay for both reverse transcription and the first round of PCR was used. Briefly, 5-μl prepared RNA solution containing either liver (1.5-μg total RNA) or serum RNA was mixed with 45 μl of PCR solution, including 1 × PCR buffer, 250 μM of each of four dNTPs, 4.0-mM MgCl2, RNAsin 10 U, 0.4 μM each of primers, 40 U of MMLV reverse transcriptase (Promega), and 1.25-U Taq polymerase (Perkin-Elmer-Cetus, Norwalk, NJ). The thermocycler was programmed to incubate samples at 37°C for 45 min, 95°C 4 min, followed by 35 cycles consisting of 94°C for 1 min, 45°C for 1 min, and 72°C for 1 min with a 7-min final extension at 72°C. For the second round of amplification, 5-μl PCR product from the first round was added to a similar reaction mixture as the first except the 4.0-mM $MgCl_2$ and outer primers were replaced by 2.5-mM $MgCl_2$ and inner primers, respectively. PCR was carried out for 35 cycles as described for the first amplification. The contamination prevention measures suggested by Kwok and Higuchi [1989] were adopted in all procedures; 20 μl of the second amplification was analyzed by electrophoresis in a 1.5% agarose gel containing 0.5 μg/ml of ethidium bromide and visualized under ultraviolet light. The size of the expected amplification product was 212 bp. The remainder of the PCR product was stored at –20°C for subsequent use in the sequencing.

Analysis was performed in duplicate for each sample. If only one of the two duplicates was positive, the test would be repeated and confirmed by using one round of the PCR amplification followed by Southern blotting, in which the PCR primers and probe were designed from NS5 region (sense, 5′-GGACTTCCGGATAGCTGARAAGCT-3′; antisense, 5′-GCRTCCACACAGATGGCGCA-3′; probe, 5′-CYCGCTGRTTTGGGGTGTACTGGAAGGC3′, where R = A or G, Y = C or T). Briefly, after running the 1.5% agarose gel loaded with 20-μl RT-nested PCR product, the DNA was transferred to Hybond-$N^+$ membrane (Amersham) by Southern blotting. The DNA was detected by electrochemiluminescence using ECL kit (Amersham). To validate the specificity of RT-nested PCR product amplified from 5′ UTR of HGV/GBV-C RNA, PCR products of three cases were sequenced directly.

PCR product was purified by Wizard PCR Preps DNA Purification System (Promega). Nucleotide sequences were directly determined using ABI PRISM dye terminator cycle sequencing ready reaction kit with Amplitaq FS on an ABI 373 A automated sequencer (Applied Biosystems, Foster City, CA). The antisense inner primer was used as sequencing primer. Semiquantitative titers of HGV/GBV-C RNA both in serum and in liver tissue were determined by serial endpoint dilution. Serial 10-fold dilutions of total RNA were reverse-transcribed and amplified by nested PCR as described above. The relative concentration of HGV/GBV-C RNA was determined by the highest dilution at which HGVGBV-C RNA remained detectable. Detection of mRNA for glyceraldehyde 3-phosphate dehydrogenase (G3PDH) was used as an internal standard with the primers (Clontech Lab, Palo Alto, CA) sense, 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′ and antisense, 5′-CATGTGGGCCATGAGGTCCACCAC-3′, with an expected RT-PCR product size of 983 bp.

For the detection of HCV RNA, the PCR primers used were chosen from the highly conserved 5′ noncoding region of the HCV genome [Shindo et al., 1992]. For external primers, the following were used: sense, nucleotides 1–21, 5′-GGCGACACTCCACCATAGATC-

Table I. Detection of HGV RNA by RT-Nested PCR in Liver Transplantation Recipients With Various Diseases

| Diagnosis | No. | HGV RNA-positive | % |
|---|---|---|---|
| Chronic hepatitis C | 11 | 2 | 18% |
| Chronic hepatitis C + alcohol | 11 | 4 | 36% |
| Alcoholic liver disease | 7 | 1 | 14% |
| Cholestatic | 9 | 2 | 22% |
| Fulminant hepatic failure | 1 | 1 | 100% |
| Cryptogenic | 3 | 2 | 66% |
| Chronic hepatitis B | 2 | 0 | 0% |
| Hepatocellular carcinoma | 2 | 2 | 100% |
| Miscellaneous | 10 | 3 | 30% |

3′, and antisense, nucleotides 304–324, 5′-GGTGCACGGTCTACGAGACCT-3′. For internal primers: sense, nucleotides 28–48, 5′-CTGTGAGGAACTACTGTCTTC-3′, and antisense, nucleotides 264–-284, 5′-CCCTATCAGGCAGTACCACAA-3′. The PCR protocol was basically the same as above for amplifying HGV/GBV-C RNA. The size of the expected amplification product was 257 bp. The relative concentration of HCV RNA was determined by the semiquantitative method described above. HCV RNA levels in serum and liver tissue were also determined using the Quantiplex HCV RNA 2.0 Assay (bDNA) (Chiron) [Alter et al., 1995]; 50 μl serum or 5 μl extracted RNA from liver tissue was used. The data from liver tissue were then normalized according to extracted RNA concentration and the weight of each liver sample.

## RESULTS

We studied serum and liver specimens from 56 patients who underwent liver transplantation for end-stage liver disease at our institution between 1993 and 1997. The patients included 37 (66%) males and 19 (34%) females aged between 20 and 73 years (mean, 47 years). The diagnoses recorded in their charts included chronic hepatitis C with and without alcoholism, alcoholic liver disease, cholestatic diseases, cryptogenic liver disease, hepatocellular carcinoma, chronic hepatitis B, fulminant hepatic failure, and other miscellaneous causes (Table I). A total of 17 patients had HGV/GBV-C RNA detectable either in liver or serum, including 11 in both serum and in liver, 5 in serum only, and 1 in liver but not in serum. In the six discordant cases, samples were retested by repeating the extraction of RNA either from another aliquot of serum or from a different portion of liver tissue. In each case, the same results were noted. Direct sequencing of HGV/GBV-C PCR products of three subjects confirmed the specificity of the method (data not shown).

Table I shows the distribution of HGV/GBV-C RNA in patients with various forms of liver disease. In comparing the HGV/GBV-C RNA-positive to -negative cases, no significant differences were found with regard to age (mean age, 45.5 vs. 47.6), gender (64.7% male vs. 61.5%) or with regard to HCV RNA detectability in either liver or serum in the same samples (29% vs. 41%). Semiquantitative estimation of the HGV/GBV-C

Table II. Semiquantitative Assay of HGV RNA and HCV RNA in Liver and Serum Among the 17 Patients Positive for HGV/GBV-C RNA in Serum or Liver

| | Patient number | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| HGV RNA | | | | | | | | | | | | | | | | | |
| Liver | 1 | 1 | 2 | 2 | 0 | 1 | 1 | 1 | 1 | Negative | Negative | Negative | 3 | 1 | Negative | Negative | 2 |
| Serum | 2 | 7 | 3 | 5 | 3 | 1 | Negative | 1 | 3 | 0 | 1 | 4 | 5 | 3 | 0 | 0 | 3 |
| HCV RNA | | | | | | | | | | | | | | | | | |
| Liver | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | 4 | 3 | 4 | 0 | 3 |
| Serum | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | 3 | 4 | 3 | Negative | 2 |

Titers were determined by serial 10-fold endpoint dilution and reverse transcription-PCR, then were converted to $\log_{10}$ as shown in this table. Among those where it was detectable, the mean titer of HGV RNA in liver was significantly lower than that in serum (1.33 vs. 2.56, $P < 0.05$). While the mean titer of HCV RNA in liver is slightly higher than that in serum (2.8 vs. 2.4, $P > 0.05$).

Table III. Comparison of HCV RNA Levels in Serum and Liver of 16 Patients With HCV Infection Alone to 5 With Both HCV and HGV Infection

| HCV RNA level | HCV only | (range) | HCV + HGV | (range) | *P* |
|---|---|---|---|---|---|
| Serum (Meq/ml) | 6.7 | (0.1–39.1) | 3.9 | (0.2–13.7) | >0.05 |
| Liver (Meq/gm) | 64.6 | (0.5–196) | 70.2 | (0.9–280) | >0.05 |

RNA titer in liver and in serum by serial 10-fold endpoint dilution revealed that the mean log titer in liver was 1.33 (range, 0–3), significantly lower than the mean log titer in serum of 2.56 (range, 0–7) ($P < 0.05$), whereas in five cases coinfected with HCV and HGV/GBV-C, HCV RNA was in slightly higher concentration in liver than serum (mean log titer, 3.0 vs. 2.8) using the same methodology. Notably, endpoint titers of both HCV and HGV/GBV-C were similar in serum, although titers of HGV/GBV-C were substantially lower in liver tissue (Table II).

Because of the large amount of liver tissues available, we were able to repeat these experiments two or three times using total RNA extracted from different parts of the liver and obtained very similar data each time. To check that the absence of HGV/GBV-C RNA within the liver in five cases where it was present in serum could not be accounted for by degradation of cellular RNA, we measured the expression of a constitutively expressed gene, G3PDH, and found it to be expressed comparably in all liver tissues. Among the 22 patients seropositive for anti-HCV, 20 had HCV RNA detectable in both liver and serum, 1 in liver only, and 1 had negative results both in serum and in liver.

Furthermore, to test for a possible inhibitory effect of HGV/GBV-C on HCV, we directly quantitated HCV RNA level both in liver and in serum by bDNA assay. No significant difference was found between the 16 cases with HCV infection only and the 5 patients coinfected with both viruses (see Table III).

## DISCUSSION

It was found that a relatively high proportion of patients with end-stage liver disease appear to be infected with HGV/GBV-C, including both those with cryptogenic liver and those with other recognized forms of liver disease. Pessoa et al. [1997] reported a similarly high prevalence of HGV/GBV-C infection in patients with cryptogenic liver disease, but found no evidence of a causal relation between HGV/GBV-C infection and liver disease. The high prevalence of HGV/GBV-C infection was also reported in the patients with extrahepatic malignancies [Toniutto et al., 1998]. Taken together, these studies suggest that HGV/GBV-C infection may not be the underlying cause of the liver injury but rather an associated infection.

The main finding in this study was that fully 30% of individuals found to be infected with HGV/GBV-C based on the presence of circulating HGV/GBV-C RNA did not appear to have hepatic infection (no HGV/GBV-C RNA detectable in liver). In only one case (5.8%) was the converse found (HGV/GBV-C RNA in liver but not in serum). Semiquantitative estimation of the relative amounts of HGV/GBV-C RNA in serum and liver showed that the titers of HGV/GBV-C RNA were 10- to 100-fold higher in serum than in liver. This is in direct contrast to the findings with HCV, a virus known to be hepatotropic where HCV RNA was more likely to be present within liver than serum and comparable titers were found in liver and serum using the same methodology. Other studies have indeed shown that titers of HCV RNA may be 10- to 100-fold higher in liver than in serum [Fong et al., 1991]. Considering the similar titers in serum of two viruses, the data confirmed that HGV/GBV-C does not appear to replicate predominantly in the liver.

Some recent studies using strand-specific PCR have found replicative intermediates of HGV/GBV-C within the infected human livers [Madejon et al., 1997; Saito et al., 1997; Laskus et al., 1998]. However, this only indicate that HGV/GBV-C may replicate in the liver and, when comparing the data with HCV [Laskus et al., 1997], it appears that the liver is not the major site of replication. These findings have implications for understanding the pathogenicity of HGV/GBV-C.

This agent was designated a hepatitis virus because it was isolated from serum derived from two individuals with blood-borne hepatitis. Studies of both post-transfusion and community-acquired hepatitis have suggested that HGV/GBV-C infection may be associated with hepatitis, which is typically mild and, while chronic infection with HGV/GBV-C is almost universal, chronic hepatitis is much less common. A recent chimpanzee inoculation study indicated that HGV could be transmitted by infected serum but was not associated with hepatitis among infected animals [Bukh et al., 1998]. Although seroepidemiological studies have identified HGV infection in patients with various forms of liver disease, including cryptogenic hepatitis, fulminant hepatic failure, and hepatocellular carcinoma, it remains unclear whether HGV infection is causative or simply associated with these conditions. Our findings that HGV does not appear to be hepatotropic supports the idea that HGV is unlikely to cause significant or severe liver disease.

## REFERENCES

Alter HJ, Sanchez-Pescador R, Urdea MS, Wilber JC, Lagier RJ, Di Bisceglie AM, Shih JW, Neuwald PD. 1995. Evaluation of branched DNA signal amplification for the detection of hepatitis C virus RNA. J Viral Hep 2:121–132.

Alter HJ, Nakatsuji Y, Melpolder J, Wages J, Wesley R, Shih JW, Kim JP. 1997. The incidence of transfusion-associated hepatitis G virus infection and its relation to liver diseases. N Engl J Med 336:747–754.

Alter MJ, Gallagher M, Morris TT, Moyer LA, Meeks EL, Krawczynski K, Kim JP, Margolis HS. 1997. Acute non-A-E hepatitis in the United States and the role of hepatitis G virus infection. N Engl J Med 336:741–746.

Berg T, Naumann U, Fukumoto T, Bechstein WO, Neuhaus P, Lobeck H, Hohne M, Schreier E, Hopf U. 1996. GB virus C infection in patients with chronic hepatitis B and C before and after liver transplantation. Transplantation 62:711–714.

Bukh J, Kim JP, Govindarajan S, Apgar CL, Foung SKH, Wages J, Yun AJ, Shapiro M, Emerson SU, Purcell RH. 1998. Experimental infection of chimpanzees with hepatitis G virus and genetic analysis of the virus. J Inf Dis 177:855–862.

Chomczynski P, Sacchi N. 1987. Single-step method of RNA isolation by acid quanidinium thiocyanate-phenol-chloroform extraction. Analytic Biochem 162:156–159.

Dickson RC, Qian K, Lau JYN. 1997. High prevalence of GB virus-C/hepatitis G virus infection in liver transplantation recipients. Transplantation 63:1695–1697.

Dussol B, Charrel R, De Lamballerie X, Berthezene P, Brunet P, De Micco P, Raoult D, Berland Y. 1997. Prevalence of hepatitis G virus infection in kidney transplant recipients. Transplantation 64:537–539.

Fong TL, Shindo M, Feinstone SM, Hoofnagle JH, Di Bisceglie AM. 1991. Detection of replicative intermediates of hepatitis C viral RNA in liver and serum of patients with chronic hepatitis C. J Clin Invest 88:1058–1060.

Francesconi R, Giostra F, Ballardini G, Manzin A, Solforosi L, Lari F, Descovich C, Ghetti S, Grassi A, Bianchi G, Zauli D, Clementi M, Bianchi FB. 1997. Clinical implications of GBV-C/HGV infection in patients with "HCV-related" chronic hepatitis. J Hepatol 26: 1165–1172.

Hwang SJ, Lu RH, Chan CY, Wang YJ, Wu JC, Lee SD. 1997. The role of hepatitis G virus infection in patients with acute posttransfusion hepatitis in Taiwan. Gastroenterology 112:1260–1264.

Kwok S, Higuchi R. 1989. Avoiding false positives with PCR. Nature 339:237–238.

Laskus T, Radkowski M, Wang L, Vargas H, Rakela J. 1997. Lack of evidence for hepatitis G virus replication in the livers of patients coinfected with hepatitis C and G viruses. J Virol 71:7804–7806.

Laskus T, Radkowski M, Wang L, Vargas H, Rakela J. 1998. Detection of hepatitis G virus replication sites by using highly strand-specific Tth-based reverse transcriptase PCR. J Virol 72:3072–3075.

Linnen J, Wages J, Zhang-Keck ZY, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter K, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Shih JWK, Young L, Piatak M, Hoover C, Fernandez J, Chen S, Zou JC, Morris T, Hyams KC, Ismay S, Lifson JD, Hess G, Foung SKH, Thomas H, Bradley D, Margolis H, Kim JP. 1996. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. Science 271:505–508.

Madejon A, Fogeda M, Bartolome J, Pardo M, Gonzalez C, Cotonat T, Carreno V. 1997. GB virus C RNA in serum, liver, and peripheral blood mononuclear cells from patients with chronic hepatitis B, C, and D. Gastroenterology 113:573–578.

Martinot M, Marcellin P, Boyer N, Detmer J, Pouteau M, Castelnau C, Degott C, Auperin A, Collins M, Kolberg J, Wilber J, Benhamou JP, Erlinger S. 1997. Influence of hepatitis G virus infection on the severity of liver disease and response to interferon-a in patients with chronic hepatitis C. Ann Int Med 126:874–881.

Nakao H, Okamoto H, Fukuda M, Tsuda F, Mitsui T, Masuko K, Lizuka H, Miyakawa Y, Mayumi M. 1997. Mutation rate of GB virus C/hepatitis G virus over the entire genome and in subgenomic regions. Virology 223:43–50.

Pessoa MG, Terrault NA, Ferrell LD, Kim JP, Kolberg J, Detmer J, Collins ML, Yun AJ, Viele M, Lake JR, Roberts JP, Ascher NL, Wright TL. 1997. Hepatitis G virus in patients with cryptogenic liver disease undergoing liver transplantation. Hepatology 25: 1266–1270.

Saito S, Tanaka K, Kondo M, Morita K, Kitamura T, Kiba T, Numata K, Sekihara H. 1997. Plus- and minus-strand hepatitis G virus RNA in liver tissue and in peripheral blood mononuclear cells. Biochem Biophy Res Comm 237:288–291.

Shindo M, Di Bisceglie AM, Biswas R, Mihalik K, Feinstone SM. 1992. Hepatitis C virus replication during acute infection in the chimpanzee. J Inf Dis 166:424–427.

Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, Desai SM, Mushahwar IK. 1995. Isolation of novel virus-like sequences associated with human hepatitis. Nat Med 1:564–569.

Tagger A, Donato F, Ribero ML, Chiesa R, Tomasoni V, Portera G, Gelatti U, Albertini A, Fasola M, Nardi G, the Brescia HCC Study. 1997. A case-control study on GB virus C/hepatitis G virus infection and hepatocellular carcinoma. Hepatology 26:1653–1657.

Tanaka E, Alter HJ, Nakatsuji Y, Shih JW, Kim JP, Matsumoto A, Kobayashi M, Kiyosawa K. 1996. Effect of hepatitis G virus infection on chronic hepatitis C. Ann Int Med 125:740–743.

Toniutto P, Pirisi M, Fabris C, Bardus P, Soardo G, Vitulli D, Tisminetzky SG, Pacco P, Gasparini V, Baralle F, Bartoli. 1998. High prevalence of infection with hepatitis G virus in patients with hepatic and extrahepativ malignancies. J Hepatol 28:550–555.

Yoshiba M, Okamoto H, Mishiro S. 1995. Detection of GBV-C hepatitis virus genome in serum from patients with fulminant hepatitis of unknown aetiology. Lancet 346:1131–1132.